Summary of Maryland's *Pfiesteria*-related Comprehensive Assessment and Rapid Response Efforts in 1998

Prepared by the Maryland Pfiesteria Study Team
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Introduction

During late summer and fall 1997, fish kill episodes and lesion events occurred on the Lower Eastern Shore of Maryland, first in the Pocomoke River and then in Kings Creek and the Chicamacomico River. During 1997, the State of Maryland mobilized rapid response and comprehensive assessment teams to protect public health and to learn more about the causes and conditions leading to the observed fish health problems. The toxic stages of *Pfiesteria pisicicida* were confirmed through scanning electron microscopy and bioassay analyses in each of the outbreak areas. A number of alternative hypotheses relating to the fish health problems have been, and continue to be, evaluated by the State's *Pfiesteria* Study Team which is coordinating the environmental studies related to *Pfiesteria*. Another important finding during 1997 was the conclusion by a medical team that measurable human health effects were associated with the outbreak areas.

During 1998, the comprehensive assessments related to *Pfiesteria* and fish health were broadened in scope on the Lower Eastern Shore region of Maryland. In addition to these new assessment activities, ongoing Bay-wide programs were selectively enhanced to collect comparative information on fish health, water quality, and other environmental conditions that could help to evaluate unique conditions that may be occurring in the Lower Eastern Shore outbreak areas. Rapid response teams were equipped with protocols to respond to fish kills or lesion events where *Pfiesteria* could be implicated. River systems were prioritized for monitoring intensity as Level I - Rapid response to fish health events, Level II - monitoring on the 3 river systems affected in 1997, Level III - monitoring of 5 more river systems with water quality characteristics similar to Level II systems, and Level IV - use of existing Bay-wide programs to support comparative efforts.

A suite of programs were involved in the comprehensive assessment for 1998. These included:

- fish health surveys for incidences of anomalies
- fish health investigations including experimental studies and pathological/microbiological assessments
- environmental assessments including water quality, habitat, *Pfiesteria*, and other biological measurements

- watershed and pollution source assessment
- toxicant assessments

Selected results from these comprehensive assessments are summarized below, followed by a more complete summary of each of these major program elements.

Results

No fish kills attributable to *Pfiesteria piscicida* occurred in 1998. Environmental conditions in the Pocomoke River preceding the fish kills in 1997 were significantly different from the same time period in 1998. Rainfall and flow differences between years changed the patterns and timing of the chlorophyll maxima and low dissolved oxygen levels between the two summer seasons, perhaps preventing ideal conditions for the concentration of menhaden and the proliferation of toxic *Pfiesteria* and/or closely related dinoflagellates.

Incidence of anomalies on fish in general, and menhaden in particular, declined between 1997 and 1998 in the waters of the Lower Eastern Shore. Despite the broad scale statistics on low anomaly incidence, two localized events of elevated incidence rates for lesions on menhaden were recorded in 1998 on the Lower Eastern Shore. Events of two to three weeks duration occurred in the Wicomico River in August and the Chicamacomico River in September. Presumptive counts on water samples collected in these areas indicated relatively low abundances ($\leq$ 130 cells/ml) of *Pfiesteria*-like cells present in each case. Bioassays of water samples collected failed to show the presence of actively toxic *Pfiesteria* although one bioassay did turn toxic after 8 1/2 weeks. New techniques in genetic identification of *Pfiesteria piscicida* conducted this year did, however, indicate the presence of *Pfiesteria* in the two times and places where elevated levels of menhaden lesions were found.

Atlantic menhaden with lesions, collected from several Chesapeake Bay tributaries, consistently showed evidence of invasive fungal disease. Experimental studies involving caged white perch held in the Pocomoke River showed the development of anomalies such as abrasions and tail rot. These anomalies became more severe the longer the fish were held. Physiological indicators of these caged fish did not show symptoms of severe stress.

Broader scale water quality evaluations of Lower Eastern Shore tributaries in 1998 reinforced the preliminary findings of 1997 that most of these rivers have relatively high nutrient concentrations. This is particularly the case for organic forms of nitrogen, phosphorus and carbon. Watershed studies are beginning to clarify the processes and pathways of nutrient and sediment delivery from the watershed to the lower rivers.

Acknowledgments

In almost all aspects of these comprehensive assessment and rapid response activities, Maryland investigators pursued active collaborations with leading researchers in the field to utilize the latest information and techniques, as well as to provide sampling and logistical support to various researchers. Maryland is indebted to these many collaborators, too numerous to mention here, and the periodic reviews provided by the Harmful Algal Technical Advisory Committee under the leadership of Dr. Donald Boesch. Maryland investigators are also grateful for the funding support provided by several federal agencies and the State legislature.

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SUMMARY
COMPREHENSIVE FISH POPULATION HEALTH (LESION) SAMPLING - 1998

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Introduction

The appearance of fish with various types of ulcers has been linked to activity by Pfiesteria piscicida a toxic dinoflagellate. The mode of activity is thought to be that the toxin erodes the slime coating and possibility the epithelia and allows the entry of fungi and bacteria which causes the ulcer. Based on recommendations from researchers, Maryland has adopted a water contact closure criteria which depends upon finding lesioned fish as an bio-indicator of Pfiesteria activity. A program was set up in the DNR Fisheries Service in 1997 to sample water bodies statewide to detect the presence of Pfiesteria from samples of fish. Atlantic Menhaden Brevoortia tyrannus are thought to be a species particularly vulnerable to the toxin. In the first year, collections of fish were primarily from existing surveys done for purposes of monitoring commercial or recreational species. Monitoring of areas closed due to Pfiesteria-caused fish kills was done as a directed activity. In 1998, staff were added and sampling was done on a directed basis in those areas at risk for Pfiesteria activity.

Objectives

1. Provide information on the incidence and distribution of fish lesions in Chesapeake Bay and Maryland's Oceanside tributaries.

2. Serve as a platform for researchers to provide healthy and diseased fish for histopathological and microbiological analysis.

3. Respond to citizen calls into the DNR Fish Health Hotline.

Methods

Sampling in 1998 for fish health, Pfiesteria, and habitat quality monitoring was done on four levels. Level I was termed Rapid Response deployments. As had been done in 1997, a 24 hour a day Fish Health Hotline was maintained by the Natural Resources Communication Center in the Tawes Building. This Hotline received calls from citizens regarding fish health problems. Fisheries Service biologists staffed the Rapid Response Team and responded to potential problems identified through the Hotline. An agreement between Maryland Department of the Environment Fish Kill Unit and Fisheries Service assured a timely response to fish kill or fish health reports.

For Level I investigations, fish communities were sampled with otter trawl, beach seine or cast net. Three water samples for Pfiesteria and three for habitat quality along with physical parameters were taken when dead fish were present or closure criteria were met (more than 20% of fish of one species with Pfiesteria-like lesions in a sample greater than 50 fish).

Level II sampling targeted those rivers which had been closed in 1997 (Pocomoke River, King's Creek and Chicamacomico River). These systems had 44 sample sites. Selected sites within these systems were sampled on 60 different days.

Level III sampling focused on water bodies with water quality and watershed characteristics similar to Level II rivers and therefore vulnerable to Pfiesteria outbreaks. There were 40 Level III sample sites on Fishing Bay, Nanticoke, Wicomico, Annemessex and Manokin Rivers. Selected sites in these systems were sampled on 114 different days.
The Chester, Elk, Potomac and Patuxent Rivers were less intensively sampled. Sixty-five sites were sampled during 29 sampling days.

Sampling methods for Levels II and III were identical and sampling days often collected data from both Level II and III sites. Bottom trawls, using a 16 foot net, were made at open water sites in each system. Sites were at equidistant river mile designations. Exact locations were selected based on site logistics and freedom from snags. One 6-minute trawl was made at each site perpendicular to the channel. If the river was too narrow to finish a six minute trawl, the boat and trawl were turned into the tide and parallel to the channel to finish the tow. Seine hauls, using a 100 foot net, were made at the nearest snag-free beach. Tributaries of each major river were sampled at access points using cast nets. Twenty casts were made with a six foot net at each site.

Catch was counted and twenty fish of each species were measured. Any abnormalities such as lesions or parasites were noted and lesions on menhaden were characterized as new, old or recovering. All individuals with anomalies were measured. At the request of fish health specialists, fish samples were retained and processed for histology, microbiology or other analysis. Water samples for analysis of Pfiesteria or nutrients were taken on request. Water quality (dissolved oxygen, temperature, pH, conductivity and salinity) was recorded at each sampling site.

For Level IV sampling, all Fisheries Service projects were asked to note the frequency and types of anomalies on fish that were collected in the various fishery dependant and fishery independent samples taken in Maryland tidewater. Projects included an angler survey on head boats out of Crisfield, the Striped Bass Juvenile Seine Survey, Striped Bass Tagging Program, Crab Trawl Survey, Bay Pound Net Survey, Coastal Bays Trawl and Seine Project, Baseline Fish Health Survey and Biological Indicators Trawl Survey.

Results

A total of 372,675 resident and migratory finfish were sampled by 8 projects from 36 water bodies in Maryland's portion of the Chesapeake Bay, and ocean-side tributaries, to monitor for the presence of anomalies or lesions believed to be Pfiesteria-related. In 1997 188,824 fish were sampled. Lesion prevalence among all fish species sampled by these programs in 1998 was 0.5%.

Atlantic menhaden was the fish of most concern since river closures in 1997, and closure criteria in 1998, were based on lesion prevalence in this species. A total of 46,789 menhaden were collected in 1998 and represented 12.6% of all fish sampled. In 1997, 16,622 menhaden were collected and accounted for 8.8% of all fish sampled.

Lesion prevalence among menhaden was lower in 1998 (2.1%) than in 1997 (9.1%) in all systems that were sampled. This trend was also seen in systems which were intensively monitored both years because of closures in 1997: Chicamacomico River, Kings Creek and Pocomoke River. In 1997 of the 5754 menhaden captured in these systems, 22.3% had lesions. In 1998, in these same waters, 3012 menhaden were captured with a 11.2% lesion prevalence.

The prevalence of lesions among menhaden collected by cast net from the Chicamacomico in September through November 1998 (n= 1783) was 15.6% compared to 69.1% in September through November 1997 (n=856). Lesion frequency also showed declines during the same time period in the other two rivers that were previously closed. King's Creek lesion prevalence among menhaden caught with cast nets in 1998 (n=318) was less than one half 1997's (n= 4719; 10.4% vs. 22.0%). Cast netting was not done in the Pocomoke in 1997, so data from all gears was combined for both years. This also showed a decline between 1997 (n= 179) and 1998 (n= 321; 77.2% vs 8.4%).

Numbers of all fish and numbers of menhaden collected per day from the Chicamacomico in 1998 were significantly greater than in 1997. September through November numbers of menhaden captured per day (95.6- 1998 vs. 13.7-1997) and catch per unit of effort (CPUE) for menhaden (2.38-1998 vs. 0.30-1997) suggest that the increase in their numbers in this system is real and not a sampling artifact. Numbers of fish per day and menhaden per day collected in King's Creek and CPUE for menhaden were similar for both years. Data from the Pocomoke is not comparable between years because different gears were used.

The Fish Health Hotline received 441 calls in 1998. A large proportion (35%) of the calls concerned striped bass displaying shallow sores and reddening of the skin. Reports describing this condition were clustered in areas of high angling activity and were made during the striped bass open season. The Fish Health Project in cooperation with Maryland’s Department of Agriculture’s Animal Health Lab identified a complex of bacteria and fungi in the sores. The causative agent has not yet been identified but Pfiesteria toxicity has been ruled out.

Fisheries Service and Department of Environment Fish Kill Unit biologists responded by phone or personal contact to 50 citizens that called in on the Hotline. Citizens were informed of the present situation and in some cases diseased fish were picked up from callers and diagnoses were attempted or samples of fish were collected by the responding biologists. Several of the calls reported menhaden with lesions and Rapid Response Teams were sent to collect menhaden and determine the proportion of lesioned fish. None of the sampling resulted in finding high numbers of lesioned menhaden.

In early May a Rapid Response Team was sent out to the Pocomoke in response to a report of diseased fish. Trawl and cast net samples on May 8, 9 and 10 found no menhaden and low levels of disease problems in other fish collected.

On August 4 a Fish Health monitoring crew reported high levels of lesions among menhaden collected from the Wicomico River. Repeated sampling by a Rapid Response crew between August 5 and 24 found up to 80% of menhaden with lesions. There was never any evidence of fish kills or erratic behavior by fish and it was felt a closure was not warranted.

On September 3, a large sample number of lesioned menhaden were found in a non-routine cast net sample from the Chicamacomico River. Daily sampling found a high proportion of lesioned menhaden. Again, there was no evidence of a fish kill or erratic behavior. Sampling continued until September 28 when the number of menhaden substantially declined. No closure was enacted.

The routine monitoring and research projects, not specifically involved in Pfiesteria sampling, collected 54% of the total fish examined. Only 0.2% those fish had any sort of cut, wound, infection or other anomaly. Of the 2582 menhaden captured, only 0.7% had any sort of anomaly. In general, Chesapeake Bay fish appear to be healthy although there may be localized or species specific problems which are not related to Pfiesteria.

SUMMARY
FISH HEALTH ASPECTS OF PFIESTERIA INVESTIGATIONS IN MARYLAND - 1988

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Introduction

A suite of fish health investigations was undertaken in Maryland's Chesapeake Bay and tributaries during 1998. One purpose of these studies was to monitor for the effects of *Pfiesteria piscicida* and other *Pfiesteri*a-like organisms, both to observe impacts on fish populations and communities, and to alert the public should outbreaks of these toxic dinoflagellates occur. A second purpose was to gain better information about the causes, progression and outcomes of the high prevalence of fish abnormalities seen in the Pocomoke River and Sound in 1997.

Toxic *P. piscicida* outbreaks occurred in the Pocomoke system and other tributaries in 1997 in association with fish abnormalities, but there remained questions about whether other factors were involved in the high incidence of fish anomalies. For example, it was clear that some of the less severe lesions seen in commercial fisheries catches were related to physical damage from confinement in traps (pound nets, fyke net and bank traps) and handling. Unfavorable environmental conditions, water quality problems, toxic discharges and epizootic fish diseases also were proposed to explain some or all of the abnormalities.

This report includes results from 1) on-board monitoring of commercial fishery catches in the Pocomoke system; 2) experimental studies conducted to assess physical and physiological responses of caged and uncaged fish exposed to the Pocomoke River and Sound environments; and 3) pathology and microbiology of ulcerated Atlantic menhaden collected from Chesapeake Bay tributaries. Information about Baywide monitoring of fish abnormalities is reported elsewhere[1].

Commercial Fishery Observations

External abnormalities of fish from Pocomoke River and Sound commercial fisheries were uncommon in 1998, in contrast to observations from 1997. Of 34,942 fish observed from pound net and fyke net catches in 1998, including 44 species, only 0.306% were observed with some form of skin anomaly and only 0.192% possessed a severe skin anomaly (erosions or ulcers). No correlation between fish size (total length) and the occurrence of skin anomalies was observed for any species. Abrasions were the most commonly observed skin anomaly, followed by erosions and ulcers. A majority of the erosions and ulcers observed were on striped bass. A condition known as ulcerative dermatitis, caused by bacterial infection, has been seen widely in striped bass populations over the past two years, and is under separate investigation. Hemorrhages and tail rot were present in some fish but were less common than other anomalies.

Experimental Studies

Seventeen *in situ* experiments were conducted from April-October 1998, to evaluate the formation and progression of skin anomalies in the lower Pocomoke River. The experimental fish (white perch and striped bass) experienced a consistent progression of common skin anomalies. Confinement of fish in the experimental cages consistently resulted in caudal fin hemorrhage (60-80% of fish). This high percentage of caudal hemorrhage also occurred in the uncaged reference fish, permitted to swim

[1]H. Rickabaugh, R. Lukacovic and H. Speir. 1999. Comprehensive Fish Health Sampling. Draft report. Maryland Department of Natural Resources. Annapolis.

freely in 1000 gallon (3900 L) holding tanks.

The percentage of fish with abrasions was generally 20-60% at all sites, including both caged and uncaged reference experiments. The white perch were abraded by the wire holding cages and physical contact with fish holding tanks. Tail rot typically occurred after 48 hours and steadily increased throughout the experiments. Tail rot was common in fish at all sites including caged and uncaged references. Skin abnormalities were least prevalent in the uncaged reference experiments, indicating that minor skin abnormalities, primarily abrasions and hemorrhage, resulted from caging white perch and striped bass. The occurrence of *severe* skin abnormalities such as erosions and ulcers was very low at all sites, consistent with observations from the nearby commercial catch.

Many fish initially had mild abrasions and caudal hemorrhage, which disappeared as they acclimated to the experimental holding conditions. Some abrasions and caudal hemorrhaging, however, progressed to fungal, viral, or bacterial infections. Abrasions induced by contract with experimental cages and holding tanks often progressed into lymphocystis or formed an erosion which would eventually progress to an ulcer. Fin and tail hemorrhage induced by handing stress and mechanical contract with experimental cages commonly progressed to tail rot. Tail rot sometimes resulted in deterioration of the entire caudal fin within 48 hours after onset and ultimately caused mortality. Although tail rot could proliferate very quickly, most fish were not severely affected and the experimental period usually ended prior to any mortality.

One of the objectives of the experimental study was to determine whether either the Pocomoke River environment or confinement in cages caused significant physiological stress in fish. Blood samples from caged and uncaged white perch were analyzed for hematocrit (red blood cell concentration), leucocrit (white blood cell concentration), plasma osmolality (roughly equivalent to salt content), plasma chloride concentration and plasma glucose concentration. A pilot study also was conducted to measure acetylcholinesterase (an important enzyme that regulates neuromuscular activity) from the brains of caged and uncaged white perch.

None of the physiological indicators showed differences between caged and uncaged fish large enough to reflect severe stress. The largest differences from mean physiological conditions generally were measured in fish freshly removed from pound nets or fyke nets. These indicators tended to return to mean values after acclimation in the holding tanks. It should be noted that no baseline data on these indicators was available for white perch prior to these studies. The values for the uncaged reference fish are the best available baseline physiology for this species.

Based on pilot work conducted in 1998, we believe that similar experiments can be done in 1999 using Atlantic menhaden, a more fragile species with direct relevance to *Pfiesteria piscicida* toxicity.

Pathology and Microbiology

Atlantic menhaden tissues from four Chesapeake tributaries, the Eastern Shore Wicomico, Annemessex, Chicamacomico, and Nanticoke Rivers, were examined by a fish pathologist. Twenty-four of 27 menhaden (89%) were diagnosed with mycotic granulomatosis, i.e. fungal infections with evidence of specific immune responses by the fish. This diagnosis is equivalent to the ulcerative mycosis thought to be associated with toxic *Pfiesteria piscicida* activity, except that the former designation does not presume that the external ulcers were caused by fungal infection. Bacterial cultures from the internal organs of these menhaden, along with three ulcerated menhaden collected from the Pocomoke River did offer evidence of epizootic bacterial disease. *Aeromonas hydrophila* and *Pseudomonas fluorescens*, ubiquitous aquatic species, were the most common isolates. Both species are fish pathogens, but are generally thought to be opportunistic, causing serious infections only stressed or injured fish.

Cultures from liver, kidney and spleen tissues from the experimental white perch also did not show evidence of epizootic bacterial disease. *Aeromonas hydrophila* was the most common isolate; this species was most prevalent in fish that had been caged for over 96h.

Conclusions

- The incidence of external anomalies on commercially harvested fish in the Pocomoke River and Sound was much lower in 1998 than in 1997.

- Atlantic menhaden with ulcers, collected from several Chesapeake tributaries, consistently showed evidence of invasive fungal disease.

- No evidence for acute toxic or pathogenic effects on fish was found either in field sampling or in the experimental deployments in the Pocomoke system during 1998.

- White perch held in *in situ* cages in the Pocomoke system for more than 48h developed abrasions and tail rot, symptoms which became more severe with longer holding times. Mild hemorrhages and abrasions were common even with shorter holding times. Physiological indicators did not show symptoms of severe stress.

- The high incidence of severe anomalies observed in Pocomoke system commercial fisheries during 1997 could not be attributed to effects of the pound nets, fyke nets and bank traps in which they were captured and confined, based on the results of 1998 caging studies and commercial fishery observations.

- The experimental systems and procedures developed during these studies can be employed effectively to monitor and identify potential acute toxic or pathogenic events, and could be used with Atlantic menhaden if great care is taken in capture and transport of the fish.

SUMMARY
FINDINGS FOR WATER QUALITY, HABITAT AND *PFIESTERIA*-RELATED ORGANISMS - 1988

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Introduction

During the summer and fall of 1997, Maryland experienced four separate toxic outbreaks of the dinoflagellate, *Pfiesteria piscicida* in three Eastern Shore rivers -- the Pocomoke River, Kings Creek, and the Chicamacomico River. These outbreaks resulted in the deaths of thousands of fish, a high percentage of fish with lesions, and negative impacts to the health of some individuals who came in contact with the affected waterways.

During 1998, the Maryland Department of Natural Resources (DNR) instituted a monitoring program on the three rivers affected in 1997 and five additional rivers that exhibit similar habitat conditions. The goals of this monitoring program were threefold: first, to protect public health by identifying potential outbreaks as soon as possible, second, to refine our understanding of the environmental factors that contribute to the growth and toxic activity of this very complex organism, and third, to track progress in improving habitat conditions.

Water and habitat quality, including several measures of the phytoplankton community, were monitored monthly at up to 8 to 12 sites in each of the eight monitored rivers, April through October. Additionally, plans were made for intensive, rapid response monitoring of water quality, and phytoplankton if and when unexplainable fish kills or high percentages of fish with lesions were found.

Preliminary Results for 1998 and Comparisons with 1997

Maryland experienced no fish kills or lesion outbreaks in 1998 similar in magnitude to the four 1997 events. During 1998, investigations were made of several limited fish health events. In two cases, a substantially higher than natural percentage of fish were observed with the type of anomalies similar to those associated with toxic *Pfiesteria* outbreaks. One event was at Shiles Creek on the Wicomico River in early August, and the other was on the Chicamacomico River (same site as 1997's outbreak) in early September. Additional fish, water quality, and phytoplankton sampling was initiated at both sites in response to the findings.

During 1997, fish bioassays and scanning electron microscopy (SEM) confirmed the presence of toxic *Pfiesteria piscicida* at the time and place of all four fish health events. To date, fish bioassays conducted with samples collected during the two 1998 events have failed to demonstrate that *Pfiesteria* was active in its toxic state. Light microscopy found low levels ($\leq$ 130 cells/ml) of a dinoflagellate similar in size to *Pfiesteria* present at both 1998 events, and researchers working on the development of molecular probes specific to *Pfiesteria* have had positive results with water samples collected at the events, suggesting that *Pfiesteria* may have been present at low densities. Toxic activity was eventually demonstrated in a fish bioassay on a sample from the Chicamacomico River after a long incubation, suggesting that *Pfiesteria* was present, but not in an actively toxic state when collections were made. Analysis by SEM to confirm *Pfiesteria's* presence is underway.

Two confirmed toxic outbreaks of *Pfiesteria* occurred on the Pocomoke River during August, 1997. Habitat quality data collected before, during, and after the outbreaks suggests that this could have been the result of a combination of nutrient enrichment, biological activity, and the physical setting. In the vicinity of Shelltown, the Pocomoke River dramatically changes from a relatively deep and narrow river to a wide, shallow, and slow moving river. This change in physical setting provides a more favorable environment (more light, less flushing) for phytoplankton to grow and utilize the high concentrations of nutrients moving downriver. A peak in phytoplankton biomass was seen in the vicinity of Shelltown during August of 1997. *Pfiesteria* feeds on algae during some of its non-toxic stages, and these elevated algal levels could have provided the food supply for large populations of non-toxic *Pfiesteria* to develop. Algae also serve as prey for menhaden, and the blooms may have acted to concentrate schools in this region as demonstrated by research in similar estuaries. Finally, a sharp drop in dissolved oxygen levels immediately upstream of Shelltown may have further concentrated menhaden in this area by restricting their movement upriver. This model suggests the possibility that a combination of 1) a large *Pfiesteria* population and, 2) dense concentrations of menhaden in a slow moving portion of the river, was ideal for *Pfiesteria* to transform to its toxic state and result in the observed fish lesions and kills during August, 1997.

Differences in the timing and amount of rainfall likely prevented the combination of factors that set the stage for toxic outbreaks in 1997 from repeating themselves in 1998. Mid winter through July 1998 was generally wetter on the Lower Eastern Shore than in 1997. As a result, Pocomoke River flow was more than two times greater from January through April, 1998 and May through the third week in July 1998 than during the same periods the previous year. These 1998 differences in flow, especially in the May through late July period, may have resulted in sufficient changes in the timing and location of algal blooms and low dissolved oxygen events to prevent a repeat of 1997's toxic outbreaks.

Flow patterns in 1997 resulted in salinities in the Shelltown region that remained at about 8 ppt from June through September, except for a brief dip in early August due to a storm event. In 1998, salinities in June started at about 2 ppt and gradually rose to 8 ppt in September. These flow patterns appear to have changed the location of the chlorophyll maxima in the river in a similar manner. The effect of flow on the positioning of chlorophyll maxima is a well accepted phenomenon in the Chesapeake Bay. Just as flows in 1997 kept salinities relatively stable, the summer chlorophyll maximum in this year remained primarily in the vicinity of Shelltown, especially in August. In 1998, the chlorophyll maximum moved from downriver of Shelltown in the spring to upriver, near Pocomoke City, in August and September. In 1998, therefore, the summer chlorophyll maximum was not in a location that would have served to concentrate menhaden in the Shelltown region, as hypothesized for conditions in 1997.

Flow patterns may also have influenced the low dissolved oxygen region upriver of Shelltown during summer. In 1998, the severity and extent of this low dissolved oxygen phenomenon was less than it was in 1997. These more favorable habitat conditions may, in turn, have reduced the "blockage" effect that was hypothesized as a factor contributing to a possible concentration of menhaden in the Shelltown region. For this type of oxygen depression, which occurred in a non-stratified water column, the higher 1998 summer flows probably lessened the severity and extent by increasing flushing rates.

In general, estuarine nutrient conditions were similar between 1997 and 1998. An exception was observed in orthophosphate concentrations. In 1997, orthophosphate was at high concentrations at and above Pocomoke City from June through September and briefly further downriver following the storm event in early August. These higher concentrations were not observed in 1998 until July and did not penetrate as far downriver in August. Again, flow was the most likely factor contributing to the interannual differences. Soils, sediments and waterbodies in the Pocomoke watershed are known to be highly enriched in phosphorus. Orthophosphate bound to soils and sediments under oxygenated conditions can be released when oxygen levels are very low. It is likely that low oxygen conditions were more prevalent in the watershed (e.g. ditches, streams) as they were in the estuary during 1997 vs. 1998. As previously discussed for the estuary, higher summer flow rates in 1998 probably reduced the severity and extent of low dissolved oxygen conditions in the watershed. The orthophosphate patterns may have influenced the phytoplankton community in general and may have stimulated *Pfiesteria* growth directly, as shown in laboratory studies.

In conclusion, differences in rainfall and flow appear to have changed a number of potentially important environmental factors between 1997 and 1998. The resulting changes to ecological dynamics in the river may have been sufficient to prevent a toxic *Pfiesteria* outbreak from reoccurring in 1998.

SUMMARY
POCOMOKE WATERSHED POLLUTION ASSESSMENT - 1988

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Background

The Pocomoke River watershed is a combined watershed that includes the drainages of the Pocomoke, Manokin and Big Annemessex Rivers. The Pocomoke River watershed drains approximately 433 sq. mi. of Worcester, Somerset, and Wicomico Counties in Maryland, 38 sq. mi. in Delaware, and 17 sq. mi. in Virginia. The primary tributaries of the Pocomoke River are Dividing (62 sq.mi.) and Nassawango(69 sq.mi.) Creeks. The Manokin and Big Annemessex Rivers drain 163 sq. mi. in Somerset and Worcester Counties, Maryland.

The land cover in the Upper Pocomoke River watershed and the Lower Pocomoke River watershed is forested and agricultural. Forest accounts for 53% of the land use in the Upper Pocomoke and 58% in the Lower Pocomoke. This is slightly less than the overall percentage of forest cover for Maryland's Lower Eastern Shore Tributary Strategy Basin, which is 61% forested. Agricultural land in the Upper Pocomoke accounts for 45% of the land use, while in the Lower Pocomoke it accounts for 36% of the land use. This is slightly higher than the overall percentage of agricultural land for Maryland's Tributary Strategy Lower Eastern Shore Basin, which is 32% agricultural. Urban land in these two subwatersheds accounts for 3% of the land use, while overall in the Lower Eastern Shore Basin, urban land accounts for 5% of the land.
The largest communities in the Pocomoke watershed are Pocomoke City, Princess Anne, Crisfield and Snow Hill.

Agriculture in the watershed is mixed cash grain, vegetable crops and livestock. Corn, soybeans, and small grains are the predominate crops. Poultry production is the main type of livestock agriculture. The annual poultry population is estimated at approximately 22,400,000. The manure produced by all of the livestock in the watershed is estimated to be in excess of 329 million pounds.

The modeled nutrient loads for these two basins are not proportional to the land use. In the Upper Pocomoke basin, 90% of the total nitrogen load is from agricultural land, followed by forested land at 6%, urban land at 2% and point source loads at less than 1 percent. Sources of loads in the Lower Pocomoke are similar to the Upper Pocomoke with 82% of the total nitrogen load from agricultural land, 8% from forested land, 3% from urban land, and 3 % from point source loads. Loads from septic systems and atmospheric deposition to open water make up the difference. For total phosphorus loads, 80% of the load is from agriculture in the Lower Pocomoke, and 97% of the load is from agriculture in the Upper Pocomoke.

In an assessment of the 138 Maryland 8-digit watersheds (of which the Lower Pocomoke River and Upper Pocomoke River are two), a variety of indicators were examined in order to prioritize watersheds for either restoration, protection, or both. These indicators covered water chemistry, aquatic living resources, and landscape characteristics. Both the Lower Pocomoke River and Upper Pocomoke River watersheds were determined to be in need of restoration. The Lower Pocomoke River watershed was also determined to be in need of protection.

In the Upper Pocomoke River watershed, 7 restoration indicators (of 11 pertaining to the watershed) were cause for concern. The most notable of these is the indicator entitled historic wetland loss. This watershed had the most acres of historic wetland loss than any other watershed in the state. 80,903 acres of historic wetlands have been lost. Historically, wetlands covered more than 85% of the watershed.

In the Lower Pocomoke River watershed, 8 restoration indicators (of 12 pertaining to the watershed) were cause for concern. The most notable include the indicators entitled SAV Abundance, SAV Habitat, and Historic Wetland Loss. This watershed received a score of "most degraded condition" for SAV Abundance. In fact, all of the MD 8-digit subwatersheds of the Lower Eastern Shore for which there was data, received this score. This watershed also received a very degraded score for SAV Habitat. The Lower Pocomoke River watershed received the second worst score for the state for the historic wetland loss indicator. 71,922 acres of historic wetlands have been lost. Historically, wetlands covered more than 70% of the watershed.

Surface Water Quality Results

Water quality monitoring conducted by MDE and DNR indicates that nutrient concentrations in the fresh water portion of the Pocomoke River system are similar from Snow Hill to the Delaware state line. Total nitrogen concentrations are consistently above 1 mg/l at all sampling sites above Snow Hill. Total phosphorus concentrations are consistently above .1 mg/l.
Concentrations vary with flow and season. Nitrogen concentrations change seasonally throughout the watershed. The highest nitrogen concentrations occur during the winter months when base flows in the ditches and streams are at their highest and the vegetation is dormant. The lowest nitrogen concentrations occur during the summer months when flows are at their lowest, plant uptake is at its peak and the potential for denitrification exists. Many ditches within the watershed support dense vegetative growth along the banks and in the channel. As a result of the growth and decay of the plant material in the ditches and streams, dissolved oxygen in the water column is consumed. Dissolved oxygen concentrations follow the same seasonal patterns as the nitrogen concentrations. Dissolved oxygen levels are highest in the winter and lowest in the late summer. As stated earlier, vegetative growth in the channels creates back water conditions during the growing season. This backwater becomes stagnant during the summer and early fall. Warm, stagnant water with an ample supply of organic material creates an optimal environment for microbial activity. This activity consumes oxygen and with a limited supply of oxygen, ultimately creates anaerobic conditions. Low dissolved oxygen concentrations measured at two small sub-watershed sites within the watershed during the summer months indicates that some of these ditches are anaerobic for extended periods

during the late summer. Microbial activity in anaerobic environments consumes nitrate nitrogen releasing nitrogen gas to the environment. Denitrification can be a dominate process in ditches on the lower Eastern Shore (Denver et al.). In conjunction with plant uptake, denitrification probably contributes significantly to the decreases in nitrate nitrogen concentrations in these ditches during the spring, summer and fall.

Phosphorus concentrations are driven by suspended solid concentrations. During storm flows it appears that elevated suspended solid concentrations are related to sediment in suspension. Suspended solid concentrations and total phosphorus concentrations are also elevated during periods of extended low flow. This may be due to anoxic conditions in the ditches and in the sediment in the ditches. These anoxic conditions may cause phosphorus and iron to be released into solution from the sediment. Once in stream the iron and phosphorus may then reform as a amorphous iron hydroxide that has adsorbed phosphate which remains suspended in solution increasing suspended solid concentrations and phosphorus concentrations (Bricker personal communication).

Nutrient loads and yields have been calculated for the two small sub-watersheds. The nutrient loads are flow driven. Loads increase with increasing flow for all constituents. The seasonal variation in nitrate nitrogen loads due to changes in concentrations that are seen in other watersheds are masked by high nitrate nitrogen loads during high flows.

Nutrient yields are the annual loads discharges from a watershed divided by the acreage of that watershed. The yields allow comparisons to be made from one watershed to the next. Compared to average yields in the literature, the nutrient yields from the small watersheds being monitored in the Upper Pocomoke are elevated (Frink 1991).

Annual Average TN and TP Yields (lbs/acre/year)

Year	North Fork of Green Run		South Fork of Green Run		Ave. For Ag Land[1]	
	TN Yield	TP Yield	TN Yield	TP Yield	TN Yield	TP Yield
1994	6.90	0.80	8.80	1.03	11.77	1.63
1995	5.55	0.27	10.08	0.62		
1996	21.93	1.48	42.81	8.52		
1997	11.98	0.65	18.89	2.50		

1. From Frink et.al.

Ground Water Quality

The surficial aquifer on the Pocomoke watershed has a high silt and clay. The sediments are poorly drained because of poorly incised steams and flat water-table gradients. In much of this area, drainage ditches have been constructed, which will act to lower the water table. In the area closer to the Chesapeake Bay in the Pocomoke River Basin, the water table is generally shallow and comprised of poorly-drained, low-permeability sediment. The majority of ground-water in surficial aquifers discharges to adjacent streams and is termed "base flow". The percentage of total stream flow contributed by base flow ranges from about 42 to 74% (Bachman and others, in press). These data are based on using hydrograph separation techniques at 20 sites with stream flow data.

Nitrate concentrations in the surficial aquifers underlying the Pocomoke River basin generally range from <0.1 to 35 milligrams per liter, with about 10 percent of the samples above the maximum contaminant level of 10 mg/L N as NO3 set by U.S. Environmental Protection agency. The median concentration was 1.1 milligram per liter. These values are based on data collected from 1976 to 1990 (Hamilton and others, p. 45).

The nitrate concentrations appear to be related to land use and the type of sediments in the aquifer and soils. The nitrate concentrations were elevated in agricultural and residential areas in zones where the aquifer consists of sandy deposits (Hamilton and others, p. 65). The sandy composition of the aquifer promotes aerobic conditions under which nitrate is stable. In agricultural and residential areas underlain by clay and silt deposits and anaerobic water, effects of human activities are less evident. The silt and clay deposits can cause lower nitrate concentrations for several reasons (Hamilton and others, p. 65). The anaerobic conditions promote denitrification. The high amounts of clay and silt can inhibit downward movement of fertilizers from the land surface into the water table and have abundant exchange sites for ammonia, which reduces the amount of nitrogen available to reach the water table.

The age of the ground-water can also affect the nitrate concentrations. Knowing the apparent age of ground water can help explain nitrogen concentrations and provide an estimate of how long a management practice may take to be effective. Based on existing data the apparent ground-water ages in the surficial Coastal Plain aquifers range from about 5 to 20 years (Focazio and others, 1998, in press).

Nutrient Budget

Nutrient inputs to the two small sub-watersheds being studied in the Upper Pocomoke are dominated by agricultural sources. On average 96% of the N inputs to these watersheds are supplied by agricultural sources. Crop acreage is roughly 40% corn, 55% soybeans and 5% small grains each year. Typically poultry litter is applied at 5 tons/acre to corn and 2.5 tons/acre to small grains. Soybeans receive no addition nutrient inputs. Total N and P inputs average 291 lbs of N/acre and 67 lbs of P/acre each year.

Annual removal of nutrients through grain harvesting is the largest export of nutrients from the watershed. Nutrient uptake ranges from 139.3 to 184.7 lbs. N / acre of cropland and 19.4 to 34.3 lbs of P/ acre of cropland in these two small watersheds.

Net nutrient inputs to the watersheds vary year to year based on crops and yields. From 1995 through 1997 N surpluses have averaged 147 lbs / acre of cropland and P surpluses averaged 47 lbs of P / acre of cropland.

<u>Watershed Modeling Results</u>

The Chesapeake Bay Program Phase IV Watershed Model (WSM) calculates point and nonpoint source pollutant inputs to Bay watershed streams and the subsequent transport of these pollutants to the Bay. The Pocomoke and Manokin Watersheds are represented by a single segment (Segment 430) in the Watershed Model. Thus one set of model inputs and outputs is used to represent these watersheds.

Land use information in the Watershed Model is based on the Chesapeake Bay Program Land Use coverage (CBPLU). The CBPLU is a combination of EMAP, CCAP, and GIRAS land use data. The Pocomoke watershed extends into Virginia (6%) and Delaware (7%), but is largely contained in Maryland (87%). The watershed in Maryland is roughly 33% agriculture, 48% forest and wetlands, 2% developed land, and 3% water. Agricultural lands are further split into conventional till, conservation till, hayland and pasture base on the 1992 Agricultural census and the Conservation Tillage Information Center.

Nutrient inputs to the model include point sources, septic systems, atmospheric deposition, and manure and chemical fertilizer applied to agricultural lands. Manure inputs to the model are based largely on the 1992 Agricultural Census. Manure is applied to agricultural lands at a rate (137 pounds N per tilled land acre and 40 pounds P per acre in the Pocomoke Watershed) that ensures all manure generated is used. An input rate of 70 pounds N per acre and 20 pounds P per acre is assumed for chemical fertilizer application to tilled land. Atmospheric N is deposited at a rate of 10 pounds per acre to all lands.

The fate of these inputs are modeled (for example, plant uptake accounts for 97 pounds N per tilled acre) by the Watershed Model resulting in land use loading rates to tidal waters given below:

	Convention (lbs/ac)	Conservation (lbs/ac)	Hayland (lbs/ac)	Pasture (lbs/ac)	Forest (lbs/ac)	Developed (lbs/ac)
Nitrogen	31.5	25	3.7	8.8	1.8	16.5
Phosphorus	2.6	1.8	0.4	0.3	0.01	0.4

These loading rates applied to the Watershed Model land use acres result in a load to tidal waters, ignoring Best Management Practice implementation, of 13.4 million pounds of N and 0.16 million pounds of P annually.

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SUMMARY
1998 MONITORING FOR TOXICS IN WATERS DEMONSTRATING TOXIC *PFIESTERIA* EPISODES: PRELIMINARY ANALYSIS OF DATA AND COMPARISON TO 1997

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Introduction

In the late summer and fall 1997, a series of fish kill episodes occurred in several water bodies in Maryland's lower eastern shore, including the Pocomoke River. The fish kills occurred in the absence of other commonly encountered causes (e.g., very low dissolved oxygen, chemical spills) in waters also demonstrating significant incidents of fish lesions (deep, bleeding, and often circular sores) considered "typical" of those associated with "*Pfiesteria*-like" organisms. In response to these episodes, the State undertook a large study to determine possible causes for the fish kills and lesions. Although scanning electron microscopy and fish toxicity bioassays conducted by other investigators subsequently confirmed the presence of toxic life stages of several dinoflagellates, including *Pfiesteria piscicida*, the State continued investigating other possible contributing factors. Current scientific knowledge suggests that the factors most likely contributing to the presence of toxic *Pfiesteria* are the natural physical characteristics of the River (especially the broad shallow mouth and deep channel in the upper river), and certain chemical characteristics, both natural (e.g., deeply colored water limiting light penetration) and anthropogenic (e.g., nutrient enrichment). However, acute and/or chronic exposure to certain chemical contaminants are known to cause lesions and kill fish and could play a potential role in the Pocomoke.

To explore toxics as a possible factor in the Pocomoke episodes, concentrations of chemical contaminants in water were evaluated during 1997 and 1998. In the fall 1997, after a 1-1.5 inch rain event, water samples were collected for analysis of a suite of pesticides with reported use in the region, as well as for analysis of trace pesticides and dissolved metals sampled and analyzed using clean technique. Pesticide samples were taken on Day #1 following the storm event, while dissolved metals samples were taken on Day #2.

In the spring 1998, water samples were collected at five locations in the Pocomoke watershed on Day #1 following each of two separate storm events (9 May and 13 June 1998). Both storm events were large enough to produce visible runoff into several tributaries of the Pocomoke River. The two river stations were also sampled a second time, on Day #3 following each storm, to determine if chemical concentrations in the Pocomoke were influenced by period of time after each runoff event.

Objectives

The objectives of 1997-1998 monitoring for toxic substances were:

To determine if chemical contaminants (agricultural pesticides and dissolved metals) are present in the Pocomoke after rain/runoff events (conditions during which pesticides due to contemporary anthropogenic sources approach a near "worst case" scenario);

To determine if levels of chemical contaminants observed in fall 1997 and spring 1998 rain/runoff events may possibly be associated with the episodes of fish kills and high fish (menhaden) lesion incidence observed in 1997 in the Pocomoke.

Methods

Grab samples of surface water were collected from the Pocomoke River and three small tributaries to the river following rain/runoff events. Agricultural pesticides receiving substantial use in the region and trace dissolved metals were target analytes. Pesticide analyses were performed by the Maryland Department of Agriculture (MDA) State Chemist Laboratory for both 1997 and 1998 sample collections. Additional trace pesticide analyses were performed by George Mason University (under contract to U.S.Geological Survey) in the fall 1997 and by the U.S. Department of Agriculture (in cooperation with University of Maryland Chesapeake Biological Laboratory) in the spring 1998. Trace pesticide analysis at George Mason University (GMU) and USDA included 14-15 of the MDA analytes (providing inter-laboratory comparison) and addressed analytical sensitivity questions for pesticides (e.g., permethrin) that are highly toxic to aquatic life. Clean technique field collection and analyses for dissolved metals was performed by the U.S. Geological Survey (USGS) in 1997. In 1998, dissolved metals were analyzed by the University of Maryland Chesapeake Biological Laboratory (CBL).

Results for 1997 and Preliminary Results for 1998

Fall 1997 and Spring 1998 Results for Pesticides : In the fall 1997, most monitored pesticides were not detected. Of the 14 pesticides found at quantifiable levels, nine were known to receive substantial usage in the watershed (acetochlor, alachlor, atrazine, chlorpyrifos, cyanazine, linuron, metolachlor, metribuzin, and simazine). The remaining five pesticides were not reported to receive substantial usage in the Pocomoke (methyl-parathion, napronamide, prometon, pronamide, and terbacil). All detected pesticides were found in low parts-per-trillion (pptr) or parts per billion (ppb) concentrations and all were within the general ranges commonly found in Maryland streams during other previous USGS fall line and MDE studies.

In the spring 1998, during the period of peak agricultural herbicide and insecticide application, pesticide levels were generally higher, with the exception of chlorpyrifos. The highest levels were found in the tributary stations immediately following runoff, while levels in the River remained at or below several parts per billion. Levels were again found to be similar to those reported elsewhere in Maryland and the Chesapeake Bay region following storm events.

None of these pesticides were found at levels suggesting acute toxicity to fish, aquatic invertebrates, or aquatic plants resident to Maryland.

Fall 1997 and Spring 1998 Results for Dissolved Metals Using “Clean Technique”: Dissolved metals results for the fall 1997 were reported by USGS in the open file report 99-57, “Organic Compounds and Trace Elements in the Pocomoke River”, as follows:

> “Initial results indicate that geochemical signatures of some of the dissolved trace elements may be unique to the Pocomoke Basin -- some of the sites sampled had much higher levels of arsenic, selenium, strontium, and lithium than those sampled in other Chesapeake Bay tributaries. At four of the five sampling locations, concentrations of dissolved arsenic ranged from 40 to 67 parts per billion and were more than two orders of magnitude higher than any concentrations measured in 1994 at the Fall Line stations of the Susquehanna, Potomac, James, Rappahannock, York, Choptank, and Nanticoke Rivers. Concentrations of dissolved selenium, strontium, and lithium also were higher by factors of approximately 100, 30, and 50 respectively, than concentrations of these elements measured in 1998 at the Chesterville Branch and Nanticoke River stations of the 1998 Chesapeake Bay Program's Fall Line Toxics Project.”

Levels of arsenic and selenium were inadvertently not determined following the spring 1998 rain/runoff events. Dissolved metals results for the inorganic analytes are summarized by CBL, in “Amended Final Report- Concentrations of Trace Metal and Pesticides in the Pocomoke River Surface Waters Following Precipitation Events 1998”, as follows:

> “Concentrations of all trace metals except copper were similar to those measured in other tributaries to the Chesapeake Bay, and do not appear to be especially enriched in the Pocomoke River system. The concentration of total copper was elevated in the tributaries on one sampling occasion. Dissolved copper concentrations were also elevated on this occasion. Measurements in the Pocomoke River itself were compromised by an analytical artifact that was not immediately recognized during sample analysis…. Because of the analytical artifacts, further investigation is required to ascertain the copper concentration in the river although the available data (e.g. particulate concentrations) suggests that concentrations are not substantially elevated in the Pocomoke River itself. While the tributary samples should not have been compromised by the analytical artifact, this assumption should also be checked.”

Conclusions and Discussion

Pesticides: Pesticide concentrations in the Pocomoke River and its tributaries after storm events are generally low, vary seasonally, and are similar to those seen in other streams and rivers in the Bay region. Direct chronic toxicity by pesticides resulting in the fish kills or high fish lesion incidence observed in the Pocomoke River also appears to be unlikely. Indirect sub-lethal effects (i.e., effects on fish immune systems in tandem with pathogenic organisms, such as *Aphanomyces* or *Pfiesteria)*, can not be eliminated from consideration at this time, based on these data. During 1999, MDE will perform an extensive search of the primary scientific literature to determine if there is any evidence associating such effects to low, transient levels of pesticides in surface waters. Since pesticide levels in the Pocomoke were similar to those seen elsewhere in the region where there have not been reports of fish kills and fish lesion incidence such as seen in the Pocomoke in 1997, it currently appears that pesticides were not a significant contributing factor to the Pocomoke's problems.

Dissolved Metals- Fall 1997: Two days following a fall 1997 storm event, USGS found that surface water samples from most of the Pocomoke stations demonstrated elevated levels of dissolved arsenic and selenium. Arsenic and selenium are contained in compounds of known poultry-feed additives and arsenic is believed to be directly excreted by chickens (see USGS report cited previously for further details). Based on reports of relatively high production estimates for chickens in the Delmarva region, it is possible that poultry operations in this region may be contributing relatively large loads of these trace metals to the environment. This premise receives some further support from the results of additional USGS analyses of sediment samples that show levels of arsenic and selenium substantially higher in the Pocomoke and lower eastern shore than levels that USGS finds in sediments in other regions. Further monitoring for these trace elements in the Pocomoke watershed therefore is clearly warranted.

Observed elevations in strontium and lithium are currently unexplained. When further monitoring for arsenic and selenium is conducted in the Pocomoke, it would be interesting to continue to measure the levels of these elements as well.

Thus far, the data for arsenic and selenium are limited to a single sampling. Although collected following a storm event, based on their field observations, USGS concluded that their sampling occurred during base or low flow conditions. Further monitoring for these two elements following storm events as well as during base flow conditions therefore appears warranted. This is especially true since some of the levels of selenium reported by USGS are above Maryland's water quality standards for selenium and some of the levels for arsenic are approaching Maryland's water quality standards levels.

Dissolved Metals- Spring 1998: With the exception of copper, trace metals analytical results for the spring 1998 storm event collections generally showed levels of metals in the Pocomoke watershed that are within the range seen elsewhere in the Bay region. This suggests that nickel, chromium, lead, zinc, mercury and cadmium were not major contributing factors to the fish kills and other incidents in the Pocomoke in 1997. Levels of copper in the upper region of the watershed also did not appear to be elevated over those seen elsewhere in Maryland, but data for both the lower river and tributary stations are difficult to interpret due to analytical artifacts.

Although sample collections for metals were carried out in both fall and spring seasons, confounding factors prevent sound conclusions from being drawn by direct comparison of the two sets of data collected during the two seasons. There are some differences in their results (e.g., CBL detected significant (order of magnitude) elevations in dissolved copper in two of their tributary stations in the spring 1998, while USGS did not see such significant elevations in the fall 1997) which could be the result of one or more of the following: seasonal and/or hydrogeological differences between the collections; use of different field and analytical methods; as yet unexplained point source or non-point source loadings.

Immediate Future Plans: In an effort to reduce at least one of these confounding factors (use of different field and analytical methods), USGS and CBL will carry out a winter 1998 side-by-side, parallel sample collection and inter-laboratory comparison. Plans as of 1/27/99 call for the collection of two additional spring storm events. It is concluded by USGS, CBL, and MDE that more dissolved metals data are needed to establish potential associations between the concentrations of at least several metals (arsenic, selenium, and copper) and effects on fish health or occurrence of pathogens, as well as the concentrations of metals and the land use and agricultural practices of the region.